Bangladesh J. Fish. Res., **1**(2) : 67-74 July 1997
Bangladesh Fisheries Research Institute

Effect of decapsulation on viability and hatching performance of *Artemia* cysts at different salinity levels

S.U. Ahmed, M.A. Rahman[1], M.N. Islam[1], and M. Kamal [1]

Brackishwater Station, BFRI, Khulna-9280, Bangladesh
[1]Department of Fisheries Technology , BAU, Mymensingh

Abstract

Artemia cysts were produced from the traditional solar salt works of Bangladesh through different fertilization treatments were tested for viability and hatching performance in different forms, such as processed and preserved, processed and decapsulated and unprocessed and undecapsulated. Decapsulated cysts performed maximum hatching (86.0%) in 20 ppt salinity during 48 hours of incubation. The hatching percentage by the unprocessed and undecapsulated cysts were very low (12.0 – 18.7%) in all the tested salinity grades.

Key words : *Artemia,* Decapsulation, Hatching, Salinity

Introduction

While feeding *Artemia* nauplii to fish and prawn larvae, the main constraint has been found to be the imperfact separation of nauplii from the hatching debris like empty shells, which often carry a heavy bacterial load (Shelbourne 1964, Gilmour *et al.* 1975). The unhatched cysts were also observed to carry a bacterial load after 24 hours of incubation (Wheeler *et al.* 1979). Such cysts upon ingested by the predator larvae cause blockage in their elimentary canal (Herald and Rackowicz 1951, Morris 1956, Stults 1974). Unhatched cysts and empty shells may also lead to serious infection in the culture system, leading to considerable mortality of the cultured larvae (Shelbourne 1964, MacFarlane 1969). Gunther and Catena (1980) reported infection of *Artemia* nauplii by pathogenic bacteria (*Vibrio* spp.) which also got transmitted to the predator larvae. A new method of feeding the *Artemia* cysts directly to the prawn larvae by decapsulating (removing of the outer chorion layer) them by sodium or calcium hyphochloride (decapsulation reagent) was found to be very useful, as it caused no harm to the viability of the embryo and also acted as a disinfectant

(Sorgeloos *et al.* 1977). Hatching efficiency and output of nauplii from decapsulated cysts were found higher than those from the untreated cysts (Bruggeman *et al.* 1980, Vanhaecke and Sorgeloos 1983). However, the effect of decapsulation has not been tested for all the strains in different environments. So it bears immense importance to find out the effect of decapsulation of different forms of locally produced *Artemia* cysts on viability and hatching performance.

Materials and methods

Artemia cysts were produced using the strain of Great Salt Lake (GSL), Utah, USA, in the *Artemia* production ponds (APP) of the modified solar salt works of Bangladesh by various feeding / manuring treatments. Treatments were T_1 (Urea and TSP at a rate of 25 and 20 kg / ha / week respectively), T_2 (dried powdered and sieved chicken manure at a rate of 125 kg / ha 3 - 4 days interval in the first year), T_3 (same as T_2 applied in the second year) and T_4 (double the rate of application of T_3 of second year). Initial fertilization was done before five days of *Artemia* nauplii release and the quantities were different than the regular ones, such as for T_1, Urea + TSP = 50 + 20 kg / ha, for T_2 and T_3, chicken manure 500 kg / ha and for T_4, chicken manure 1000 kg / ha.

Hatching of both decapsulated and undecapsulated cysts were tested in different hatching conditions :

- Hatching of the cysts in different salinity media : 10, 20, 30 and 40 ppt.
- Observation of the hatching performances of the decapsulated cysts at different time intervals, such as 24, 36 and 48 hours.
- Hatching of the cysts in different comparable forms like processed and preserved (not decapsulated), processed and decapsulated and unprocessed and undecapsulated (preserved).

The number of nauplii produced per 100 full cysts were counted and the hatching percentage was calculated following Sorgeloos *et al.* (1986) :

$$\text{Hatching percentage (HP)} = \frac{\tilde{n} \times 100}{\tilde{c}}$$

Where, $\tilde{c}$ = mean number of cysts after one hour of initial incubation.
$\tilde{n}$ = mean number of nauplii.

First produced *Artemia* cysts were processed according to Sorgeloos *et al.* (1986). The steps of processing were : a. size separation with brine, b. density separation in brine, c. washing in freshwater, d. density separation in freshwater and e. drying.

Cysts were decapsulated following the techniques described by Sorgeloos *et al.* (1977) and Bruggeman *et al.* (1979 and 1980).

The cysts hydrated up to 2 hours in fresh water at ambient temperature ranging between 25 - 28°c (hydration time increased with decreasing temperature and increasing salinity). For hydration, 1 g dried cysts wereprovided

per 20ml of water and aeration was made at a rate of 0.51 / min. Prolonged hydration prior to hypochloride treatment was avoided because it could drastically affect the hatching rate and efficiency of the decapsulated cysts.

Commercial grade sodium hypochloride (NaOCl) was diluted to 50% with sea water and 40% sodium hydroxide and was used for decapsulation. NaOH was added to increase p^H above 10.0.

Hydrated cysts were transferred to the decapsulation reagent (15 ml for 1 g cyst) and stirred well continously with a glass rod for about 10 minutes. Temperature was maintained below 40^0C by keeping the decapsulation container in cold water bath. Cysts were decapsulated within 15 minutes which were immediately filtered out on a 120 µm mesh cloth.

After complete dissolution of the chorions, the decapsulated cysts were filtered off and excessively washed on a 120 µm screen with tap water until no more chlorine smell was noticed. Hypochloride residues adsorbed into the decapsulated cysts were deactivated by dipping the cysts in 0.1N HC1 for several times.

This deactivation lasts less than one minute and then cysts were again washed with tap water. Hypochloride residues were detected by putting some decapsulated cysts in a small amount of diluted starch-iodine reagent (i.e. starch, potassium iodide, sulphuric acid and water).

After the completion of the deactivation and washing procedures, cysts were drained on a 120 µm sieve and transferred into a saturated brine solution at a rate of 1 g (decapsulated cysts) / 10 ml. Since upon incubated in brine, dehydrated cysts were releasing water so that brine had to be renewed after each hour of incubation. Settled cysts in the bottom were filtered through 120 µm screen. Cysts were then poured in fresh brine solution and kept in refrigeration for future use.

Results

Processed and preserved cysts

The cysts produced in T_3 showed highest hatching percentage (78.3% ± 4.0) in 48 hours of incubation for processed and preserved form in natural day light. The cysts of the same source offered 60.0% ±1.7 and 75.7% ±2.5 in 24 and 36 hours of incubation, respectively (Table 1). In this experiment, the cysts from all the four sources ($T_1 - T_4$) showed their best hatchability (72 – 78%) at 20 ppt salinity media. The lowest hatchability was recorded at 40 ppt salinity compare to other concentrations in 24, 36 and 48 hours of incubation. The second highest percentage (75.7%) of hatching was observed in 36 and 48 hours of incubation for the cysts of T_3 at 20 ppt and for the cyst of T_1 at same salinity.

Table 1. Percentage (mean ±SD, n = 5) of hatching of processed and preserved cystts (in natural day light)

Incub. period	Treatments	Salinity of hatching media (ppt)			
		10	20	30	40
24 hr	T_1	50.3 ±3.1	60.7 ±3.5	45.0 ± 3.0	33.7 ±2.1
	T_2	55.3 ±5.7	58.0 ±5.0	40.0 ± 5.7	32.0 ±3.0
	T_3	55.3 ± 1.2	60.0 ±1.7	50.7 ± 0.6	45.0 ±1.7
	T_4	45.0 ± 2.6	62.7 ± 0.6	50.3 ± 2.3	38.7 ± 1.5
36 hr	T_1	65.0 ± 2.6	72.0 ± 3.0	51.3 ± 2.1	40.3 ± 1.2
	T_2	65.7 ± 3.2	70.3 ± 3.8	45.7 ± 3.5	40.3 ±3.2
	T_3	64.3 ± 2.1	75.7 ± 2.5	58.7 ± 1.5	47.7 ± 2.1
	T_4	58.3 ±1.5	70.0 ± 1.7	57.3 ± 3.2	42.7 ± 0.6
48 hr	T_1	65.3 ± 1.2	75.7 ± 1.5	55.0 ±2.3	45.0 ± 1.7
	T_2	75.0 ± 3.6	75.3 ± 4.2	55.0 ± 7.2	48.7 ±5.7
	T_3	70.3 ± 1.5	78.3 ± 4.0	62.0 ± 4.4	50.7 ± 0.6
	T_4	56.3 ± 1.5	72.7 ± 0.6	54.0 ± 1.7	38.7 ± 1.5

Processed and decapsulated cysts

In case of processed and decapsulated cysts, the highest hatching percentage value (86.0% ± 4.6) was also offered by the cysts produced through T_3 in 48 hours of incubation. In 36 hours of incubation, the cysts of same source offered 84.0% ±3.5 hatchability (Table 2) which is the second highest hatching value for that particular batch. Maximum hatchability by the cysts of all sources found at 20 ppt salinity followed by 10 ppt in 36 and 48 hours of incubation. Gradual decrease in hatching percentage with the increase in salinity concentration of the hatching media was observed in all the cases. The lowest percentage of hatching (but not poor) mostly in between 57 - 67% was observed at 40 ppt salinity in 36 and 48 hours of incubation.

Unprocessed and undecapsulated cysts

In this trial, cysts from all the sources showed very minimum percentage of hatching at all the salinity grades (10, 20, 30 and 40 ppt) and in all incubation periods (24, 36 and 48 hours). Minimum hatching percentage (12.0% ±0.0) recorded for the cyst produced through T_3 at 40 ppt salinity in 36 hours of incubation and the highest percentage (18.7%) was recorded for the cyst of T_1 at 10 and 20 ppt salinity (in 48 hours of incubation) and for the cyst produced through T_3 at 20 ppt salinity in 48 hours of incubation (Table 3). However, from 20 ppt to higher concentrations, decreasing in hatching percentage of the cysts from all sources has been observed.

Table 2. Percentage (mean ±SD, n = 5) of hatching of processed and decapsulated cysts (in natural day light)

Incub. period	Treatments	Salinity of hatching media (ppt)			
		10	20	30	40
24 hr	T_1	55.0 ± 3.6	59.3 ± 4.9	53.0 ± 7.5	47.3 ± 5.9
	T_2	58.3 ±3.2	68.0 ±3.5	60.0 ± 2.0	56.0 ± 2.6
	T_3	57.0 ±5.2	63.3 ±3.1	52.0 ± 2.0	50.3 ±3.5
	T_4	53.3 ±0.6	73.0 ±2.0	59.0 ±2.6	47.0 ±2.0
36 hr	T_1	73.7 ±7.6	78.0 ±2.1	65.3 ±2.1	63.0 ±4.0
	T_2	71.0 ±2.6	77.0 ±2.6	68.7 ±3.1	64.0 ±2.0
	T_3	68.0 ±4.0	84.0 ±3.5	63.0 ±3.6	58.7 ±4.6
	T_4	59.0 ±1.0	77.7 ±4.2	67.3 ±3.2	51.7 ±1.2
48 hr	T_1	59.0 ±2.3	80.0 ±8.7	69.0 ±4.0	67.3 ±2.1
	T_2	76.7 ±9.1	81.3 ±1.2	70.3 ±1.2	66.0 ±0.6
	T_3	74.4 ±2.3	86.0 ± 4.6	64.0 ±3.5	60.0 ±1.7
	T_4	65.0 ± 2.0	79.7 ± 3.2	67.3 ± 2.1	57.7 ± 1.2

Discussion

Among all the cysts, the decapsulated ones offered highest hatchability (86.0% ±4.6) at 20 ppt salinity in 48 hours of incubation. The variation in percentage of hatching of the cysts of first two forms, such as processed and preserved and preocessed and decapsulated of all treatments were found very minimum. Hatching percentage of the cysts of these two forms was very good in 10 and 20 ppt salinity for the cysts of all sources. But for other concentrations (30 and 40 ppt) hatching was found to decrease gradually. Least hatching percentage was recorded for the cysts of unprocessed and undecapsulated cysts. Finding of higher hatchability at 20 ppt in two forms is in disagreement with the findings of Versichele and Sorgeloos (1980). According to them, maximum hatchability can be obtained at sea water (35 ppt) or at 5 ppt concentration. As cyst production, its quality and hatching performance is strongly determined by environmental conditions (Browne *et al.* 1984), nature and availability of food particles in culture environment (Lavens *et al.* 1986), but not by the gene type (Browne *et al.* 1984). So it is not unlikely to evolve such information on hatching of the same cysts in new environment of culture and feeding. Versichele and Sorgeloos (1980) and Lavens and Sorgeloos (1984) studied the influence of abiotic and/or biotic factors on the hatchability of *Artemia* cysts and found a direct correlation with the environmental factors, which is supported by the present findings also. Cysts produced through different treatments, such as chemical fertilizer and organic manure did not show any remarkable variation in hatching, which means that in all the

treatments, feeding quality has been maintained properly. Because food quality available to the reproducing adults appears to be a parameter of primary importance in determing the hatching quality of encysted offered by two types of under quality feeding to the population (Lavens and Sorgeloos 1984). However, detail information regarding optimum set of abiotic conditions for the hatching of a particular strain is still lacking (Sorgeloos 1980).

Table 3. Percentage (mean ±SD,n = 5) of hatching of unprocessed and undecapsulated cysts (in natural day light)

Incub. period	Treatments	Salinity of hatching media (ppt)			
		10	20	30	40
24 hr	T_1	15.7 ±0.6	17.0 ± 1.0	15.3 ± 0.6	13.0 ±1.0
	T_2	16.7 ±0.6	16.7 ±0.6	15.3 ±0.6	13.7 ±0.6
	T_3	16.3 ±3.0	17.3 ±1.2	16.0 ±1.0	13.0 ±1.0
	T_4	15.3 ± 0.6	16.0 ± 1.0	14.0 ± 1.0	12.0 ±0.6
36 hr	T_1	16.0 ±1.0	17.0 ±1.7	14.3 ±0.6	13.3 ± 0.6
	T_2	16.0 ±1.0	17.0 ±1.0	15.7 ± 1.5	14.3 ± 0.6
	T_3	18.0 ± 1.0	17.0 ± 1.0	15.3 ±0.6	12.0 ±0.0
	T_4	17.0 ±1.0	16.7 ±0.6	13.3 ±0.6	13.0 ±1.0
48 hr	T_1	17.7 ± 1.5	18.7 ± 0.6	15.7 ± 0.6	15.0 ±1.0
	T_2	16.7 ± 0.6	18.0 ± 0.0	16.0 ± 1.0	15.3 ±0.6
	T_3	18.3 ±1.2	18.7 ±0.6	16.0 ± 1.0	13.3 ± 0.6
	T_4	17.3 ± 1.2	17.0 ± 1.0	14.0 ± 1.0	13.3 ± 0.6

Less amount of hatching percentage as offered by the unpreserved and undecapsulated cysts in quite normal. The main causes of which as identified were : degradation of cysts by hydration, non-wintering of cysts at least for few weeks and infection of the cysts by pathogenic bacteria etc. As the cysts were not processed and preserved and not decapsulated, so the state of quality of the cysts remains in a matter of doubt. Because removal of intra-cystic water from the cysts and reduced to less than 10% is a vital factor for preventing embryonic metabolism and long term presrvation (Voronov 1974, Sorgeloos *et al.* 1976, Dempster and Hanna 1956, Clegg 1962 and 1967, Vanhaecke and Sorgeloos 1982) and wintering of cysts at -4^0C (stop metabolism) or at -25^0C (for 32 weeks) is a process of diapause deactivation of the cysts of GSL origin (Lavens *et al.* 1986) and / or a treatment with 3% H_2O_2 for 15 minutes could offer a better hatchability (Mathias 1937, Bogatova and Shmakova 1980, Bogatova and Erofeeva 1985). Therefore, the absence of these attempts in the above forms of cysts, quality was undoubtedly degraded because of embryonic metabolism and / or infection that caused mortality to the cysts embryo.

References

Bogatova, I.B., Z.1. Erofeeva, 1985. Incubation of *Artemia salina* L. diapause eggs without preliminary stimulation (using hydrogen peroxide). *Gidrobiol. Zh,* **21** (2) : 52-56.

Bogatova, I.B., Z.I. Erofeera, 1980. Activation of diapause eggs of *Artemia salina. Gidrobiol. Zh.*, **16**(3) : 108-110.

Brownee, R.A., S.A. Sallee, D.S. Grosch, S. Segreti, S.M. Purser, 1984. Partitioning genetic and environmental components of reproduction and lifespan. *Artemia* Ecology.,**65 :** 949 - 960.

Bruggeman, M. E., E. Baeza-Mesa, P. Bossuyt, P. Sorgeloos, 1979. Improvement in the decapsulation of *Artemia* cysts. *In :* Culture of Fish Fry and its Live Food. EMS Spec. Publ. No. 4. E. Stycz - Jurewicz, T. Backiel, Jaspers, G. Persoone, (eds.). Inst. Mar. Sci. Res. Bredene, Belgium.

Bruggeman, E., P. Sorgeloos, P. Vanhaecke, 1980. Improvement in the decapsulation system of *Artemia* cysts.*I n :* The Brine Shrimp *Artemia.* Vol. 3. Ecology, Culturing, Use in Aquaculture. G. Persoone, P. sorgeloos, O. Roels, E. Jaspers, (eds.). Universa Press. Wetteren, Belgium, 456 pp.

Clegg, J.S, 1962. Free glycerol in dominant cysts of the brine shrimp *Artemia salina* and its disappearance during development. *Bio. Bull.* **123 :** 295 - 301.

Clegg, J.S., 1967. Metabolic studies on cryptobiosis in encysted embryos of *Artemia salina. Com. Biochem. Physiol.* **20 :** 801 - 809.

Dempster, R.P., G.D. Hanna, 1956. Preserving *Artemia* cystts in high vacuum. *J. Aquacult.* **27** (3) : 112 - 113.

Gilmour, A., M.F.; Mc Callum, M.C. Allan, 1975. Antibiotic sensitivity of bacteria isolated from the canned eggs of the Californian brine shrimp, *"Artemia salina". Aquaculture,* **6** (2) : 221 - 223.

Gunther, D.C., A. Catena, 1980. The inetraction of *Vibrio* with *Artemia* nauplii.. *In:* The Brine Shrimp *Artemia.* Vol. I. Morphology, Genetics, Radiobiology, Toxicology. G. Persoone, P.Sorgeloos, E. Roels, O.Jaspers, (eds). Universa Press, Wetteren, Belgium, 345 pp.

Herald, E.S., M. Rackowicz, 1951. Stable requirements for raising sea horses. Aquacult J. , **22 :** 234 - 242.

Lavens, P., P. Sorgeloos, 1984. Controlled production of *Artemia* cysts under standard conditions in a recirculating culture system. *Aquacultural Eng.*, **3 :** 221 - 235.

Lavens, P.., W. Tackaert, P. Sorgeloos, 1986. Review on the cryptobiotic states of *Artemia* cysts and its diapause deactivation. In : *Artemia* research and its application. Vol.3. P. Sorgeloos, D. Bengtson, A. Decleir, E. Jaspers, (eds.). Universa Press, Wetteren, Belgium.

Mathias, P, 1937. Biologie descrustaces phyllopodes. *Actual Sci. Ind.* **441 :** 1 - 107.

Morris, R, W., 1956. Home aspects of the problem of rearing marine fishes. *Bull. Inst. Oceangr.* Monaco. 61 pp.

Shelbourne, J.E. 1964. The artificial propagation of marine fish. Advances in Marine Biology -2. F.S. Russel (ed.) : Academic press, New youk, USA.

Sorgeloos, P.., M. Baeza - mesa, F. Benijts, G. Persoone, 1976. Research on the culture of brine shrimp *Arrtemia salina,* L at the State University of Ghent, Belgium, pp 473-495. Proc. 10th Eur. Symp. Mar. Biol. Vol. I. G. Persoone and E, Jaspers (eds). Universa Press, Wetteren, Belgium, 620 pp.

Sorgeloos, P., M. Baeza – mesa, G. Claus, G. Vendeputte, F. Benijts, E. Bossuyt, G. Persoone, D. Versichele, 1977. *Artemia salina* as live food in aquaculture. Fundamental Applied Research on Brine Shrimp *Artemia salina,* L in Belgium. Eur. Mar. Cult. Soc. Spec. Publ. No. 2. E., Jaspers, P. Persoone, (eds). EMS, Bredene, Belgium, 110 p.

Sorgeloos, P., 1980. The use of brine shrimp *Artemia* in aquaculture *In :* The Brine Shrimp *Artemia.* Vol.3. Ecology, Culturing, Use in Aquaculture. G., Persoone, P. Sorgeloos, O. Roels, P. Jaspers, (eds.). Universa press, Wetteren, Belgium. 456 pp.

Sorgeloos, P., P. Lavens, W. Leger, D. Tackaert,,(eds). 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. State University of Ghent. Belgium. 319 pp.

Stults, V.J, 1974. Nutritional value of brine shrimp cysts : Encysted eggs of *Artemia salina.* Thesis. Michigan State University. Diss. Abstr., **75 :** 7262, 110 pp.

Vanhaeck, P., P. Sorgeloos, 1982. International study on *Artemia.* XVIII. The hatching rate of *Artemia* cysts – A comperative study. *Aquacult. Eng.,* **1**(4) : 263 - 273.

Vanhaecke, P., P. Sorgeloos, 1983. International study on *Artemia.* XIX. Hatching data for 10 commercial source of brine shrimp cysts and re-evaluation of the "Hatching efficiency" concept. *Aquaculture.,* **30** (1 / 4) : 43 - 52.

Versichele, D.,P. sorgeloos, 1980. Controlled production of *Artemia* cysts in batch culture : *In :* Brine shrimp *Artemia.* Vol 3. Ecology, Culturing, Use in aquaculture. G. Persoone, P. Sorgeloos, E. Roels, O. Jaspers, (eds). Universa press, Wetteren, Belgium,231 - 246.

Vornov, P.M., 1974. Influence of temperature upone viability of *Artemia salina* eggs. Zool. Zh. **53** (4) : 545 - 550.

Wheeler, R., A.I., W.H. Yudin, Clark, Jr. 1979. Hatching events in the cysts of *Artemia salina. J. Aquacult.* **18 :** 59 - 67.